International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue3, 2014, pp279-282
http://www.bipublication.com

# Implementation of Biotechnology to Overcome the Challenge of Bacterial Antibiotic Resistance by Natural Sources

**Gajanan K. Arbade[1]*, Rameshwar S.Kuptekar[2] and Mangesh N. Kothari[2]**

[1]Research scholar at Defence institute of advanced technology, Girinagar, Pune, Maharashtra India-411025
[2]MGM's College of CS and IT, Nanded, Maharashtra India-431605
*Corresponding author: Email: gajananarbade@gmail.com Tel: + 91-20-24304377

[Received-19/04/2014, Accepted-01/07/2014]

**ABSTRACT:**
Microorganisms develop the resistance against various antibiotics. This is today's major problem of various pharmaceutical industries. This necessitated the need for a continued search for new antimicrobial compounds. Understanding the mechanism of resistance is important in the development of strategies to solving the problem. There are different mechanisms by which microorganisms develop antibiotic resistance like alteration in target site, changing the enzyme structure etc. In present studies we tried to elaborate the synergistic effect of plant extracts to modify the present antibiotics. Bacteria like *Bacillus subtilis* , *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, developing the resistance against antibiotics like penicillin, Ampicillin, Erythromycin, Chloramphenicol, Levofloxin, Ofloxicin ciprofloxacin etc. There are some plant compounds which enhances the activity of antibiotics to kill the resistance microorganisms. Antimicrobial activity of plant extracts sample like *Adathoda zeylanica, Ocimum sanctum, Hibiscus rosa sinensis, Azadirachta indica, Withania somnifera and Punica granatum* were evaluated with antibiotics susceptible and resistant microorganisms. Among utilized plant extracts *Ocimum sanctum, Withania somnifera, Azadirachta indica* and *Punica granatum* show interesting results. These plant extracts show synergistic effect with antibiotics. These plant extracts can be used as antibiotic activity enhancer.

**Keywords:** Antibiotic resistance, Plant extracts, synergistic effect, antibiotics modifying agents

## [I] NTRODUCTION

The generic terms to refer to either antibiotics or chemotherapeutic agents are antimicrobic or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents [2].Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the effects of antibiotics. Antibiotic resistance evolves via natural selection

acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange [3]. The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal. Antibiotics like Penicillin and Erythromycin, which used to be one-time miracle cures are now less effective because bacteria have become more resistant. Antibiotics themselves act as a selective pressure that allows the growth of resistant bacteria within a population and inhibits susceptible bacteria. Antibiotic selection of preexisting antibiotic resistant mutants within bacterial population was demonstrated in 1943 by the Luria Delbruck experiment [6].

Resistance to antimicrobials is as a result of three main strategies namely enzymatic inactivation the drug modification of target sites and extrusion [7]. Chemical modifications could be significant in antibiotic resistance, exclusion from the cell of unaltered antibiotic represents the primary strategy in denying the antibiotic, access to its targets and this is believed to enhance resistance even in cases where modification is the main mechanism [1]. Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health [4].

Why to study antibiotic resistance: Antibiotic resistance is an important tool for genetic engineering. By constructing a plasmid which contains an antibiotic resistance gene as well as the gene being engineered or expressed, a researcher can ensure that when bacteria replicate, only the copies which carry along the plasmid survive. This ensures that the gene being manipulated passes along when the bacteria replicates. The most commonly used antibiotics in genetic engineering are generally older antibiotics which have largely fallen out of use in clinical practice. Research into antibiotic resistance has not only focused on the development of new drugs, but also on determining the mechanisms by which resistant strains emerge and how to limit their spread. The Problem of antibiotic resistance can be reduced by an ounce of prevention, by reducing widespread use, use of specific antibiotics, by using the complete dose. Some plants extract shows effects like antibiotic activity enhancers. There are many medicinally important plants extracts like *Adathoda zeylanica, Ocimum sanctum, Hibiscus rosa sinensis, Azadirachta indica, Withania somnifera and Punica granatum* works as an antibiotic activity enhancers.

## [II] MATERIALS AND METHODS

### 2.1 Plant material and extract preparation:

Plant materials used in this study are consisted of leaf extracts of *Adathoda zeylanica, Ocimum sanctum, Hibiscus rosa sinensis, Azadirachta indica, Withania somnifera and Punica granatum* from MGM College campus garden, Nanded. Methanolic extracts were produced as described previously [8]. Plant materials were dried in an open air protected from direct exposure to sunlight, and 30 g of dried plant materials were separately powdered and extracted with 80% methanol (Qualigens, Mumbai) by using the Soxhlet apparatus.

### 2.2 Antibiotics:

1) Ampicillin (Cipla pharmaceuticals, Mumbai Batch No.AL9448, Mfg Date-12, 2009, Exp. Date-11, 2011). 2) Erythromycin (Alembia pharmaceutical, Baddi, Hariyana Batch No.820-63229M Mfg Date-July 2008 Exp Date-Jun 2010). 3) Chloramphenicol (Piramal Health Care, Bhatauli Kurd, Himachal Pradesh Batch No.PXA 9031 Mfg Date-Oct 2009 Exp Date- Sep.2012). 4) Penicillin (Piramal Health Care Ltd. Ahemadabad Batch No.VC721 Mfg Date- March 2010 Exp Date-Aug-2011).

**2.3 Bacterial strains: *Bacillus subtilis*** (MTCC441), *Escherichia coli* (MTCC 739), *Staphylococcus* aureus (MTCC 96,) and *Pseudomonas aeruginosa* (MTCC2488)

**2.4 Determination of MIC of Antibiotics:**

Minimum Inhibitory Concentration (MIC) of each antibiotic is determined by plating the bacterial strains on Nutrient agar (HIMEDIA, Mumbai) plates containing different concentrations of antibiotics from 100 µg/ml to 1000 µg/ml. Resistant bacterial strains were isolated inhibition from the existing cultures in the laboratory from MTCC (Microbial type Culture Collection) by disk diffusion method and zones were determined in accordance with procedures of the National Committee for Clinical Laboratory Standard (NCCLS, 1999).

2.5 Antimicrobial tests: Resistant bacterial strains were evaluated for synergism assays. Antibacterial activity was measured using a well diffusion method following the National Committee for Clinical Laboratory Standard, NCCLS 1999 [5]. Briefly, Petri plates containing 20 ml of Nutrient agar medium were inoculated with a 24 h culture of the bacterial strains. Three wells (labeled as A,B and C) were punched by using borer in each agar plate and filled with 3 µl of plant extracts or antibiotics and in case of synergism 3 µl of each has been added into well. The controls were kept by adding only antibiotics. Triplicates of each plate have been done. Concentrations of antibiotics used in this experiment are based on MIC of the antibiotic. The plates were incubated at 37 ° C for 24 h. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well.

## [III] RESULTS

The synergistic effect of leaf extracts of medicinal plants and antibiotics on the antibiotic resistant bacterial strains was noted in the form of diameter of the zone of inhibition of the bacterial growth. The results were noted in the table 1. It was observed that the synergistic effect is effectively inhibit the bacterial growth than the alone antibiotics or plant extracts.

Among utilized plant extracts *Ocimum sanctum,Withania somnifera, Azadirachta indica* and *Punica granatum* show interesting results. These plant extracts show synergistic effect with antibiotics. These plant extracts can be used as antibiotic activity enhancer.

## [IV] DISCUSSION AND CONCLUSION

Even though pharmacological industries have produced a number of new antibiotics in last few decades, but the resistance to these antibiotics by microorganism has increased. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drug in future is still uncertain. Therefore the action must be taken to reduce this problem. E.g. To control the use of antibiotic develop research to better understand the genetic mechanism of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drug to patient. The best solution for the global problem of antibiotic resistance in pathogenic bacteria- isolation and characterization of new antimicrobial compounds from variety of sources including medicinal plants. For the studies of these experiment MICs of four antibiotics viz. penicillin, ampicillin, erythromycin and chloramphenicol and then after synergistic effect was observed. Some of the bacterial cultures from our college laboratory were screened for antibiotic resistance and some of them show positive results.

Among six utilized plants *Ocimum sanctum, Withania somnifera, Azadirachta indica* and *Punica granatum* show more activity as antibiotic activity enhancer. From this work we can concluded that plant extract can be used as antibiotic activity enhancer as it show the positive results for most of the utilized medicinal plants. Path forward for the further growth of this experiment we can isolate individual plant

compounds like alkaloids, Flavones, flavonoids, terpanoids etc. and effect on antibiotic activity enhancement can be observed.

## ACKNOWLEDGEMENT

1. Guide: Mr. Kuptekar R.S. for continuous support. 2. Prof. Kothari M.N, Vice Principal and Head of the Department, MGMS College of CS & IT, Nanded. For his experimental knowledge.

## REFERENCES

1. Davies J (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science. 264:375-382.
2. Gene Mayer (2010). Microbiology and Immunology, University of south Corollina School of medicine.
3. Hawkey PM. (1998). The origins and molecular basis of antibiotic resistance. BMJ; 317:657-60.
4. Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant Origin. Janick J (ed.), Perspectives on new crops and new uses, pp.457-462
5. National Committee for Clinical Laboratory Standard (NCCLS, 1999).
6. Soulsby E J (2005). Resistance to antimicrobial in humans and animals BMJ 331(7527) 1219-20.PMID 1630 8360 PM1289307.
7. T. Sibanda, and A. I. Okoh, (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents African Journal of Biotechnology Vol. 6 (25), pp. 2886-2896.
8. Yamuna Devi M, Wesely EG, Johnson M, (2012). Chromatographic Studies on the Tannins of Aerva lanata (L.) Juss. Ex Schultes. IOSR Journal of Pharmacy, Vol. 2, Issue 1, pp. 041-051).

| RBS | Plant Extract/ AB | Penicillin | | | Ampicillin | | | Erythromycin | | | Chloramphenicol | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AB | AB+PE | PE | AB | AB+PE | PE | AB | AB+PE | PE | AB | AB+PE | PE |
| *S.aureus* | *Adathoda zeylanica* | - | - | - | 0.8 | 1.4 | - | 0.8 | 1.0 | - | - | - | - |
| | *Ocimum sanctum* | - | - | - | 0.6 | 1.0 | 0.2 | 0.6 | 0.6 | - | 0.8 | 0.8 | - |
| | *H. rosa sinensis* | 0.4 | 0.8 | - | 0.8 | 1.2 | 0.4 | 0.4 | 0.7 | 0.4 | 0.6 | 0.9 | - |
| | *Azadirachta indica* | 0.7 | 1.0 | - | 0.5 | 0.8 | - | 1.2 | 1.8 | 0.4 | 0.8 | 1.0 | 0.4 |
| | *Withania somnifera* | - | - | - | 0.6 | 0.6 | - | 0.8 | - | - | 1.0 | 1.2 | - |
| | *Punica granatum* | - | - | 0.4 | 1.2 | 1.5 | 0.6 | 0.4 | 0.6 | - | 0.8 | 0.7 | - |
| *P.auroginosa* | *Adathoda zeylanica* | 2.0 | 1.8 | - | 0.8 | 1.0 | - | NSR | | | NSR | | |
| | *Ocimum sanctum* | 1.5 | 1.5 | - | 0.6 | 0.6 | 0.2 | | | | | | |
| | *H. rosa sinensis* | 1.8 | 2.0 | - | - | - | - | | | | | | |
| | *Azadirachta indica* | 1.5 | 1.7 | 0.8 | 1.2 | 1.6 | 0.8 | | | | | | |
| | *Withania somnifera* | 1.5 | 1.8 | - | 0.8 | 0.8 | - | | | | | | |
| | *Punica granatum* | 1.4 | 1.7 | - | - | - | - | | | | | | |
| *B.subtilis* | *Adathoda zeylanica* | 2.0 | 1.9 | - | 0.8 | 0.8 | | NSR | | | NSR | | |
| | *Ocimum sanctum* | 1.4 | 2.0 | - | 0.5 | 1.0 | | | | | | | |
| | *H. rosa sinensis* | 1.5 | 1.8 | 0.3 | 1.0 | 0.9 | 0.5 | | | | | | |
| | *Azadirachta indica* | 1.8 | 2.0 | - | 1.0 | 1.2 | 0.5 | | | | | | |
| | *Withania somnifera* | 1.6 | 1.8 | - | 1.4 | 1.4 | 0.4 | | | | | | |
| | *Punica granatum* | 1.6 | 1.8 | - | 1.0 | 1.4 | 0.2 | | | | | | |
| *E.coli* | *Adathoda zeylanica* | NSR | | | NSR | | | 1.0 | 1.2 | - | NSR | | |
| | *Ocimum sanctum* | | | | | | | 1.2 | 1.0 | 0.4 | | | |
| | *H. rosa sinensis* | | | | | | | 1.8 | 2.0 | | | | |
| | *Azadirachta indica* | | | | | | | 2.0 | 2.0 | - | | | |
| | *Withania somnifera* | | | | | | | - | - | - | | | |
| | *Punica granatum* | | | | | | | 1.3 | 1.5 | 0.6 | | | |

**Table 1**. showing the activity of antibiotics, plant extract and combine: RBS= RESISTANT BACTERIAL STRAIN, PE=PLANT EXTRACT, AB=ANTIBIOTIC, NSR=NOT SHOW RESISTANCE.